

Appl. No.: 10/535,763
Amdt. dated July 6, 2009
Reply to Office action of January 5, 2009

Amendments to the Specification:

Please replace the paragraph that bridges pages 25-26 with the following paragraph.

Intein-mediated trans-splicing of proteins with restoration of their activity is known in the prior art and is described in detail in many publications. Protein affinity interaction and/or trans-splicing can be achieved by using engineered inteins. Inteins were first identified as protein sequences embedded in-frame within protein precursor and excised during the protein maturation process (Perler *et al.*, 1994, *Nucleic Acids Res.*, 22, 1125-1127; Perler, F. B., 1998, *Cell*, 92, 1-4). All information and catalytic groups necessary to perform a self-splicing reaction reside in the intein and two flanking amino acids. The chemical mechanism of protein splicing is described in detail by Perler and colleagues (1997, *Curr. Opin. Chem. Biol.*, 1, 292-299) and by Shao & Kent (1997, *Chem. Biol.*, 4, 187-194). Inteins usually consist of N- and C-terminal splicing regions and a central homing endonuclease region or small linker region. Over 100 inteins are known so far that are distributed among the nuclear and organellar genomes of different organisms including eukaryotes, archaebacteria and eubacteria (www.neb.com/neb/inteins.html Perler, F. B., 2002, InBase, the Intein Database, *Nucleic Acids Res.* 30, 383-384). It was shown that inteins are capable of trans-splicing. The removal of the central homing endonuclease region does not have any effect on intein self-splicing. This made possible the design of trans-splicing systems, in which the N-terminal and C-terminal fragments of an intein are co-expressed as separate fragments and, when fused to exteins (protein fragments that are ligated together with the help of the Intein), can perform trans-splicing *in vivo* (Shingledecker *et al.*, 1998, *Gene*, 207, 187-195). It was also demonstrated with N- and C-terminal segments of the *Mycobacterium tuberculosis* RecA intein, that protein trans-splicing can take place *in vitro* (Mills *et al.*, 1998, *Proc. Natl. Acad. Sci. USA*, 95, 3543-3548). This phenomenon was also identified for DnaE protein of *Synechocystis* sp. strain PCC6803 (Wu *et al.*, 1998, *Proc. Natl. Acad. Sci. USA*, 95, 9226-9231). Two different genes located more than 700

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Kb.p. apart on opposite DNA strands encode this protein. It was also shown that two intein sequences encoded by those genes reconstitute a split mini-intein and are able to mediate protein trans-splicing activity when tested in *Escherichia coli* cells. An intein of the same origin (DnaE intein from *Synechocystis* sp. strain PCC6803) was used to produce functional herbicide-resistant acetolactate synthase II from two unlinked fragments (Sun *et al.*, 2001, *Appl. Environ. Microbiol.*, 67, 1025-29) and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Chen *et al.*, 2001, *Gene*, 263, 39-48) in *E. coli*.